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## CODEINE AND MORPHINE IN PAPAVER SOMNIFERUM GROWN IN A CONTROLLED ENVIRONMENT

By H. L. TOOKEY, G. F. SPENCER, M. D. GROVE, and W. F. KWOLEK

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### *Abstract*

*Alkaloids in the terminal capsule of M-89 variety of P. somniferum rise and plateau as the plant matures in a controlled environment. Lancing immature capsules does not increase yield of codeine plus morphine in the above-ground plant, but lancing may translocate alkaloids upward to the capsule. Poppies produced a high proportion of codeine relative to morphine. This effect is probably due to some environmental condition imposed.*

### *Introduction*

Several authors have studied the variation of alkaloids through the growth stages of the opium poppy or the accumulation of morphine in the poppy capsule (MIRAM and PFEIFER, 1959; PFEIFER and HEYDENREICH, 1962; BUNTING, 1963). Some of the problems encountered in extrapolating results from the literature to a specific variety or climate have been discussed earlier (TOOKEY et al., 1975). By growing plants in a controlled environment where lighting, temperature, etc. are defined, some of the variables of field-grown poppies may be eliminated and the capabilities of the plant studied under more reproducible conditions. Notable of this type of work is the study of MIKA (1955) on environmental temperatures and that of GENTNER et al. (1975) on photoperiod requirements.

As an extension of our previous work, we have studied the accumulation of morphine, codeine, and papaverine in terminal capsules, and noted the effects of removing latex on the yield of alkaloids in the upper part of the poppy plant. Quite unexpectedly, we have grown plants with a high proportion of codeine to morphine in their capsules. This result raises interesting possibilities for producing more codeine by manipulation of *Papaver somniferum* L.

## Materials and Methods

### Growth of plants

White-seeded *P. somniferum* variety M-89 (TOOKEY et al., 1975) was planted in sterilized sandy loam in 13-cm unglazed pots. Seedlings were thinned to one plant per pot at 4 weeks of age. The plants were grown in a Percival Model PG 108<sup>1</sup> chamber under conditions outlined in Table I. Plants were watered as dictated by stress, hence the amounts shown in the table are approximate. The supporting bench was successively lowered to keep the topmost leaves 46–71 cm below the lamp barrier. Fertilizer was applied in water at 1, 4, 7, 10, and 13 weeks. Each application was 0.145 g per pot of Ra-pid-gro (23% N, 19% P<sub>2</sub>O<sub>5</sub>, 17% K<sub>2</sub>O). The total nitrogen applied was an approximation of 125 kg N per hectare. Flowering was initiated by increasing day length from 8–1/2 to 14 hr after 10 weeks (MIKA, 1955). Temperatures were arbitrarily chosen, but they do represent reasonable approximations of the mean temperature limits in Arizona during the winter growing season (TOOKEY et al., 1975).

Table I  
Growth conditions for *P. somniferum* M-89<sup>a</sup>

Age of plant (weeks)	Water (ml/pot/day <sup>b</sup> )	Lighting <sup>c</sup> (hr)		Temperature (° C)	
		Photoperiod	Full lights	Day (8.5 hr)	Night (15.5 hr)
1–6	50	8.5	8	20 <sup>d</sup>	18
7–10	75	8.5	8	20	18
11	85	14	12	22	18
12	105	14	12	24	18
13	140	14	12	27	18
14–16	200	14	12	27	18
17	170	14	12	27	18
18	120	14	12	27	18
19	85	14	12	27	18
20	50	14	12	27	18
21	30	14	12	27	18

<sup>a</sup> Relative humidity was uncontrolled, it ranged 45–58% at 20° C and 24–43% at 27° C (full lights).

<sup>b</sup> Approximate volumes.

<sup>c</sup> Lights were programmed to give ca. 22 hectolux incandescent for the photoperiod; fluorescent lamps were added to give 540 hectolux for full-light period, and 270 hectolux for an intermediate period.

<sup>d</sup> Temperature given is that of a shaded thermometer on the plant bench read with full lights.

<sup>1</sup> The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

### *Harvesting*

For the maturation study, 10 terminal capsules (or flowers) plus 10 cm peduncle were harvested at 8-day intervals beginning at flower opening. Sampling of the 60 plants was blocked, based on chronological order of flowering. The first six terminal flowers to open were assigned at random to the six harvest times; the next six flowers were again assigned at random to the six harvest times, and so on. Thus each harvest time sample included three early, four mid, and three late flowering individuals. Capsules not analyzed immediately after harvest were frozen until assay. Because all terminal capsules were used for analysis, lateral capsules were cut at appropriate times after their flowering and oven dried to estimate percentage dry solids.

For the lancing study, alternate plants in the chamber were assigned to the lanced or control group before flowering. Both groups of 10 plants were well spread among early and later flowering plants. All capsules (terminal and lateral) in the lanced group were incised (multiple diagonal cuts, 0.5 mm deep) 10 days after the terminal flower opened. They were incised again 3–5 days later. All lancing was done 5 hr after the start of photo period to minimize effects caused by diurnal variation (FAIRBAIRN and WASSEL, 1964). After drying overnight, the exuded latex was collected but kept separate for each plant. Capsules (plus 10 cm peduncles) of both lanced and control groups were harvested when the capsules were mature and dry. The remainder of the above-ground plants (stem plus leaves) was harvested separately.

For a study of possible genetic selection, capsules were harvested as described for the control group of the lancing study.

### *Alkaloid assay*

Alkaloids were extracted, converted to trimethylsilyl (TMS) derivatives, and analyzed by gas-liquid chromatography (GLC) (TOOKEY et al., 1975). Mass spectral analyses were carried out as described by GROVE et al. (1976). Capsules were combined in groups of 1 to 4 for assay as required; stem and leaves were analyzed on an individual plant basis. Partial decomposition and extraneous components interfered with the quantitation of thebaine and narcotine; consequently, only codeine, morphine, and papaverine are fully reported herein.

## *Results and Discussion*

### *Maturation of terminal capsules*

Poppies grown in the controlled environment were smaller than this same variety (M-89) field grown in Arizona (TOOKEY et al., 1975) but of normal appearance (Fig. 1, photo). Terminal flowers opened over a 10-day span which was centered in the 15th week of growth. Terminal capsules expanded rapidly after flowering, attained maximum size in 8–16 days, and reached maturity by 40 days after flowering (Fig. 2).

Terminal capsules (plus 10 cm peduncles) were selected for the maturation study because they usually are the largest and contain the most morphine (SARKANY et al., 1963). By restricting the samples to terminal capsules, it is possible to harvest at a specified time after flowering without the cutting of other capsules from the same plant biasing the results.

Morphine content of early, mid, and late flowering capsules from a single maturity stage did not differ significantly from each other ( $P > 0.05$ ). Morphine

Fig. 1. Poppy plants grown in controlled environment; *P. somniferum* var. M-89 (Photo).

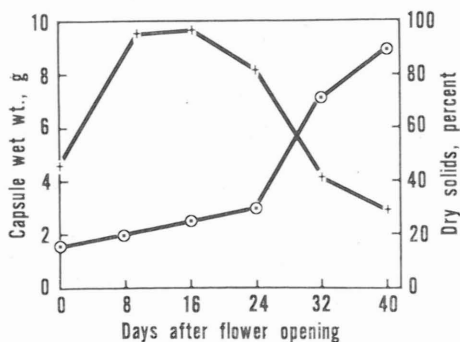
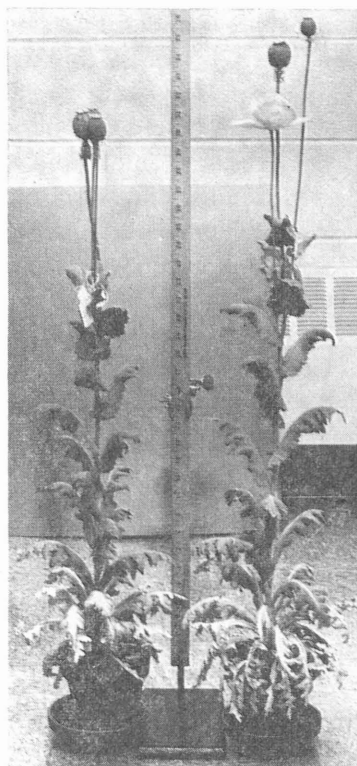


Fig. 2. Growth and maturation of poppy capsules. For details see text. +—+—+, wet weight of capsule; ○—○—○, percent dry weight.

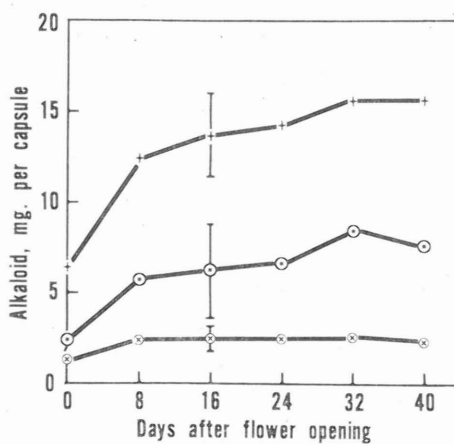


Fig. 3. Alkaloid content of poppy capsules as a function of maturity. Length of vertical lines designates the confidence limits per point ( $P < 0.05$ ) calculated from an analysis of variance. +—+—+, morphine; ○—○—○, codeine; ⊕—⊕—⊕, papaverine.

content rises rapidly at first and then levels off as the capsule approaches a mature, dry state (Fig. 3). At this stage (40 days after flowering) morphine constituted 0.60% of the capsule dry weight, including seed. The present results fail to support our previous preliminary observation of field-grown terminal capsules in which a sharp increase in morphine was seen near maturity (TOOKEY et al., 1975). The present results, however, are in agreement with the data of BUNTING (1963) on field-grown poppies. By converting BUNTING's data from percentage morphine to weight of morphine per capsule, one can show that morphine content reaches a maximum or has leveled off about the time the capsule is mature and that it gradually declines thereafter.

Codeine and papaverine content (Fig. 3) parallel that of morphine. At maturity, codeine was 6.6 mg per capsule or 0.26% of the dry weight; papaverine was lower at 2.2 mg per capsule. The codeine to morphine (C/M) ratio was 0.42 at maturity. An analysis of variance showed no evidence of the C/M ratio changing with time of harvest or with early *vs.* late flowering.

To assure ourselves that the C/M ratio was correct, mass spectral (MS) analysis of the GLC peaks was carried out. The total ionization current from automatic repetitive scanning is shown in Fig. 4 for the GC-MS output of a sample of 40-day-old capsules. Also shown are plots of intensities of ions at *m/e* ratios of 429 and 371 *vs.* scan numbers. These ions represent the molecular ions of TMS morphine and codeine, respectively. To ascertain if the peak that extends from scan 79 to 91 is TMS codeine, these 12 MS scans were summed by computer to produce the MS shown in Fig. 5. The presence of weak intensities at *m/e* 429 and

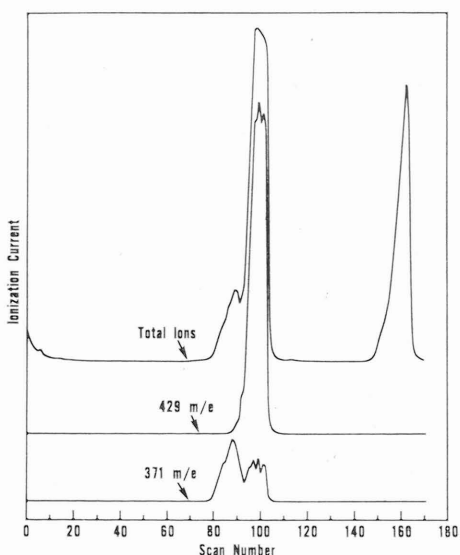


Fig. 4. Mass spectral analysis of GLC output. Intensities of individual ions calculated by computer.

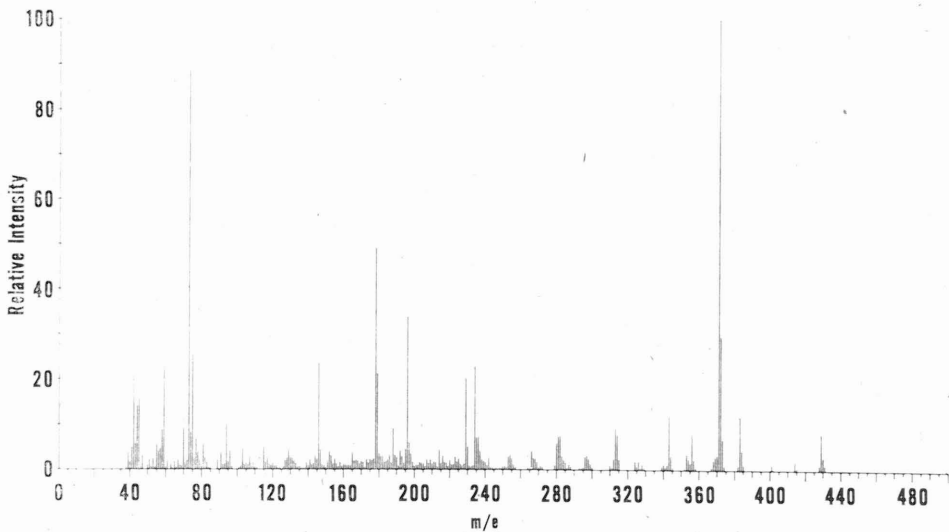


Fig. 5. Composite mass spectrum of codeine peak (scan # 79—91 of Fig. 4).

383 indicates a small amount of TMS morphine overlap from the morphine peak and an unknown component. However, this MS has a pattern like that of authentic TMS codeine treated in the same manner. The ratio of the intensity of the ion at  $m/e = 371$  to total ions is 0.096 for the TMS codeine peak from the capsules; this compares favorably with the corresponding value of 0.105 obtained from authentic codeine. Similar comparisons show that the peak from scan number 92 to 106 (Fig. 4) is essentially TMS morphine. The ion at  $m/e = 371$  (14% of base peak) is an integral part of the MS of TMS morphine (GROVE et al., 1976), but some overlap from the codeine peak cannot be ruled out.

#### *Effect of lancing*

The yield per plant of the alkaloids codeine and morphine are shown in Table II. Lancing the capsules alters the alkaloid content of the various plant parts. As FAIRBAIRN et al. (1974) have noted, stem latex is translocated into the capsule; hence a removal of latex from the capsule should affect alkaloid content in the stem. If one considers the capsules with exuded latex included as the equivalent of the intact capsule, then lancing significantly increases the yield of codeine from 5.84 mg/plant to 10.14 mg/plant ( $P < 0.05$ ). Morphine yield from the capsules likewise increases from 10.97 mg/plant to 14.12 mg/plant ( $P < 0.05$ ). However, if the entire above-ground plant is considered, lancing does not increase the

Table II  
Effect of lancing capsules on the codeine and morphine content of poppy plants

Alkaloid source <sup>a</sup>	Codeine (mg/plant)	Morphine (mg/plant)	Total (mg/plant)
Capsule:			
Latex	5.96	9.48	15.44
Remaining capsule	4.17	4.65	8.82
Lanced (sum)	10.14 <sup>b</sup>	14.12 <sup>b</sup>	24.26 <sup>b</sup>
Control	5.84	10.97	16.81
Leaf and stem:			
Lanced group	3.51	2.36 <sup>b</sup>	5.87 <sup>b</sup>
Control group	3.75	8.94	12.69
Plant total:			
Lanced group	13.65 <sup>b</sup>	16.48 <sup>b</sup>	30.13
Control group	9.59	19.91	29.50

<sup>a</sup> All plant parts (except latex) were harvested when mature and dry.

<sup>b</sup> Significantly different ( $P < 0.05$ ) from the corresponding control.

amount of alkaloids. Whereas total codeine is increased from 9.59 mg to 13.65 mg, total morphine declines from 19.91 mg to 16.48 mg. Disappearance of morphine has been noted before by FAIRBAIRN and WASSEL (1963) and should not be attributed to morphine conversion back to codeine (STERMITZ and RAPOPORT, 1961) but perhaps to normorphine (MILLER et al., 1973) or to nonalkaloidal material (FAIRBAIRN and WASSEL, 1963).

Since codeine is a precursor to morphine (STERMITZ and RAPOPORT, (1961), it is reasonable and instructive to combine these two alkaloid values. The total of morphine and codeine per plant is unchanged by lancing (cf. 29.50 mg in the control to 30.13 mg in the lanced); that is, the alkaloids which are mobilized upward by the exudation of latex from incised capsules are not replaced in the stem or leaf by new alkaloid. The precursor to codeine, thebaine, is more difficult to assess because of analytical problems noted. Nevertheless, the apparent thebaine content in stem and leaves from lanced plants (3.13 mg/plant) differs little from that of the control plants (2.36 mg/plant), and thus does not affect the conclusion regarding codeine and morphine.

#### *Abundance of codeine relative to morphine*

*P. somniferum* M-89 field-grown in Arizona produced capsules that contained codeine and morphine in a ratio of 0.077 (TOOKEY, unpublished). Latex from these poppies was analyzed in the present study and found to have a C/M ratio of 0.044. These values indicate that morphine is, as expected, the predominant

alkaloid. For example, MIRAM and PFEIFER (1959) reported data for mature capsules where  $C/M = 0.032$ .

However, M-89 plants grown in our controlled environment (Fig. 3) bear terminal capsules with a high proportion of codeine ( $C/M = 0.42$ ). Lancing the capsules (Table II) raises the  $C/M$  ratio somewhat from 0.532 to 0.718 (including latex) for all capsules ( $P < 0.05$ ). Likewise the  $C/M$  ratio for the above-ground plant is increased from 0.482 to 0.828 ( $P < 0.05$ ) by lancing. A second generation was raised from seed from M-89 plants selected for a high  $C/M$  ratio. Codeine/morphine ratios are shown in Table III. No pattern of change is apparent. At this time we do not know the reason for our relatively high codeine values. The possibility of genetic selection is not likely because of the variation between the generations in Table III. Furthermore, a single red-flowered *P. somniferum* grown in the environmental chamber also produced codeine-rich capsules,  $C/M = 0.599$ . A more likely cause of the codeine abundance is some environmental influence present in the controlled environment but not present in field culture. Suppression of the biosynthetic conversion of codeine to morphine is not defensible since  $C/M$  remains constant ( $P > 0.05$ ) throughout the maturation of the capsule (Fig. 3).

Table III  
Codeine abundance relative to morphine in all capsules of two generations of M-89 plants

Plant	C/M <sup>a</sup> ratio in capsules	
	First generation	Second generation
a	0.76	0.83
b	1.06	0.34
c	1.35	0.98

<sup>a</sup> Codeine to morphine.

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